

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification:**

Paragraph beginning at line 2 of page 7 has been amended as follows:

Figure 1. Amino acid alignment of the deduced amino acid sequence of the KCNQ5 splice variants KCNQ5-1 (SEQ ID NO:4) and KCNQ5-2 (SEQ ID NO:5). Identical residues are shaded and amino acid positions are given at the left margin.

Paragraph beginning at line 5 of page 7 has been amended as follows:

Figure 2. Amino acid alignment of KCNQ5 (SEQ ID NO:4) with human KCNQ2 (SEQ ID NO:14) (Charlier *et al.*, *Nat. Genet.* 18:53-55 (1988) and KCNQ4 (SEQ ID NO:15) (Kubisch *et al.*, *Cell* 96:437-446 (1999)). Identical residues are shaded and numbers at the left margin indicate amino acid position.

Paragraph beginning at line 28 of page 58 has been amended as follows:

A 1.15 kb clone from the middle of KCNQ5 was amplified from human brain cDNA. The sense primer was (1) 5'-CCACGTCTGCACTCAGGAAGTCTCCG (SEQ ID NO:6) and the antisense primer was (2) 5'-CCAGCTTGATTCTATGGACTGTACC (SEQ ID NO:7). The complete 3' end of KCNQ5 was amplified by standard 3' RACE PCR techniques from human brain cDNA in two successive rounds. In the first round the gene specific primer used was (3) 5'-GAAGAGCCGAGAGAAAATAACAGCAG (SEQ ID NO:8). This reaction was reamplified with the gene-specific oligo (4) 5'-

GCCCTGTGGATAGCAAAGATCTTTCG (SEQ ID NO:9) to obtain a 1.2 kb fragment that contained the entire 3' region of the KCNQ5 mRNA.

Paragraph beginning at line 3 of page 59 has been amended as follows:

The 5' end of KCNQ5 was amplified from human brain cDNA using 2 nested rounds of a standard 5' RACE PCR. The gene specific oligos used in the first and second amplifications were (5) 5'-GCTGTGAGCATAAACCACTGAACCC (SEQ ID NO:10) and (6) 5'-CCATGCGCACCATGCGGAGGATCTG (SEQ ID NO:11), respectively. A 650 bp fragment containing the missing 5' end of the KCNQ5 coding region was isolated from the second reaction.

Paragraph beginning at line 8 of page 59 has been amended as follows:

The entire coding region of KCNQ5 was then isolated in a single fragment using oligonucleotides overlapping the KCNQ5 coding sequence ends as determined from sequence analysis of the above fragments. The oligonucleotides were (7) 5'-CTCTGAATTCCACCATGAAGGATGTGGAGTCGGG (SEQ ID NO:16) 5'-(7) CTCTGAATTCCACCATGAAGGATGTGGAGTCGGG (sense) and (8) 5'-AATGTCTAGAATGGCTAAAGAACTGCTATGCCTGG (SEQ ID NO:17) 5'-(8) AATGTCTAGAATGGCTAAAGAACTGCTATGCCTGG (antisense). The first oligonucleotide includes the initiator methionine and first 20 coding nucleotides of the KCNQ5 gene. Upstream are an EcoRI restriction enzyme site for subcloning into plasmid vectors and a Kozak consensus sequence to boost translation. All nucleotides corresponding to KCNQ5 are in bold type. The second oligonucleotide is from the 3' untranslated sequence of KCNQ5 and includes an XbaI restriction site for subcloning. Non-KCNQ5 sequences at the 5' end of each primer were included for expression vector construction, but these sequences are not necessary for the amplification of the KCNQ5 gene. Only those sequences shown in bold type, which are from KCNQ5 itself, are

JEGLA, Timothy James  
Application No.: 09/810,796  
Page 7

PATENT

needed to amplify KCNQ5 when using these particular primers. The preferred template for the amplification is first strand cDNA made from some part of the human brain, or whole brain. Whole human brain cDNA was used for this reaction.

SF 1278031 v1